Circulating DNA from Plasma

User manual

NucleoSpin® Plasma XS

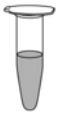
April 2007 / Rev. 01

MACHEREY-NAGEL

Protocol-at-a-glance (Rev. 01)

Circulating DNA from Plasma

NucleoSpin® Plasma XS	XS	High Sensitivity protocol	Rapid protocol

0	*Optional*		Spike addition	Spike addition
1	Prepare Sample		Use up to 240 µl plasma	Use up to 200 µl plasma
1a	*Optional: Proteinase K treatment*		Add 20 µl Proteinase K mix incubate at 37°C for 10 min	/
2	Adjust DNA binding conditions		Add 360 µl BB	Add 300 µl BB
3	Mix sample		Invert tube 3x vortex 3 sec spin down briefly	Invert tube 3x vortex 3 sec spin down briefly
4	Bind DNA		30 sec 2,000 x g 5 sec 11,000 x g	30 sec 11,000 x g
5	Wash and dry silica membrane		1st 500 µl wash buffer WB 30 sec 11,000 x g 2nd 250 µl wash buffer WB 3 min 11,000 x g	1st 500 µl wash buffer WB 30 sec 11,000 x g 2nd 250 µl wash buffer WB 3 min 11,000 x g
6	Elute DNA		20 µl Elution Buffer 30 sec 11,000 x g	20 µl Elution Buffer 30 sec 11,000 x g
7	Removal of residual ethanol		8 min 90°C	/

MACHEREY-NAGEL GmbH & Co. KG ● Neumann-Neander Str. 6-8 ● D-52355 Düren ● Germany
Tel.: +49 (0) 24 21 969 270 ● Fax: +49 (0) 24 21 969 279 ● e-mail: tech-bio@mn-net.com

Table of contents

1 Kit contents 4

2 Product description 5

2.1 The basic principle 5

2.2 About this user manual 5

2.3 Kit specifications 5

2.4 Handling of sample material 7

2.5 Elution procedures 7

2.6 Removal of residual traces of ethanol for highest sensitivity 8

2.7 Stability of isolated DNA 9

3 Storage conditions and preparation of working solutions 10

4 Safety instructions – risk and safety phrases 11

5 Protocols 12

5.1 High Sensitivity protocol for the isolation of DNA from plasma 12

5.2 Rapid protocol for the isolation of DNA from plasma 15

6 Appendix 17

6.1 Troubleshooting 17

6.2 Ordering information 18

6.3 Literature 18

6.4 Product use restriction / warranty 21

1 Kit contents

	NucleoSpin® Plasma XS		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740900.10**	**740900.50**	**740900.250**
Binding buffer BB	4.5 ml	22 ml	110 ml
Wash buffer WB	10 ml	2 x 25 ml	250 ml
Elution buffer	5 ml	5 ml	13 ml
Proteinase K (lyophilized)*	6 mg	30 mg	2 x 75 mg
Proteinase Buffer PB	0.8 ml	1.8 ml	8 ml
NucleoSpin® Plasma XS columns (plus collecting tubes)	10	50	250
2 ml collecting tubes	20	100	500
User manual	1	1	1

Reagents and equipment to be supplied by the user

- Thermal heating block
- Centrifuge for microcentrifuge tubes
- Manual pipettors and disposable pipette tips
- Microcentrifuge tubes (1.5 ml)

* For preparation of working solutions and storage conditions see section 3.

2 Product description

2.1 The basic principle

The **NucleoSpin® Plasma XS** kit is designed for the efficient isolation of circulating DNA from human blood plasma. Fragmented DNA as small as 50 – 1000 bp can be purified with high efficiency. Due to a special funnel design the **NucleoSpin® Plasma XS columns** allow very small elution volumes (5 - 30 µl) which results in highly concentrated DNA.

The protocol follows state-of-the-art bind-wash-elute procedures: After mixing of a plasma sample with the binding buffer, the mixture is applied to the **NucleoSpin® Plasma XS column**. Upon loading of the mixture DNA binds to a silica membrane. Two subsequent washing steps efficiently remove contaminations and highly pure DNA is finally eluted with 5 – 30 µl of a slightly alkaline elution buffer of low ionic strength (5 mM Tris-HCl, pH 8.5).

2.2 About this user manual

The manual provides two procedures differing in the number of handling steps, speed and performance. The **High Sensitivity procedure** is recommended if highest DNA yield and concentration is required. The **Rapid procedure** is recommended if shortest preparation time is required.

Experienced users of **NucleoSpin® Plasma XS** may refer to the Protocol-at-a-glance instead of this user manual. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. First-time users are strongly advised to read this user manual.

2.3 Kit specifications

- The **NucleoSpin® Plasma XS** kit is recommended for the isolation of fragmented cell-free DNA from human EDTA plasma.
- The **NucleoSpin® Plasma XS** kit is designed for high recovery, especially of fragmented DNA in a range of 50 – 1000 bp.
- Up to 240 µl of plasma can be used as sample material. DNA yield strongly depends on the individual sample, but is typically in the range of 0.1 to 100 ng DNA per ml of plasma.
- Elution can be performed with as little as 5 – 30 µl elution buffer. DNA is ready to use for downstream applications like real-time PCR or others.

- The preparation time is approximately 15 - 30 min for 6 - 12 plasma samples.

Table 1: Kit specifications at a glance

	NucleoSpin® Plasma XS
Sample size	Up to 240 µl EDTA plasma
Average yield	Typically in a range of 0.1 – 100 ng DNA per ml plasma, depending on the sample*. * Depending on kind of patient samples yield can be much higher
Elution volume	5 - 30 µl
Time/prep	High Sensitivity procedure: 22 – 27 min / 6 preps Rapid procedure: 15 – 20 min / 6 preps
Spin column type	NucleoSpin® XS columns

DNA yield from human plasma

DNA amounts from less than 0.1 ng DNA per ml of plasma up to several 100 ng DNA per ml of plasma have been reported (Chiu *et al.* 2006; Chun *et al.* 2006; Fatouros *et al.* 2006; Lazar *et al.* 2006; Rainer *et al.* 2006; Rhodes *et al.* 2006; Schmidt *et al.* 2005).

The content of DNA in plasma obviously depends on: condition of the donor, sampling and handling of the blood, plasma preparation and DNA isolation method, DNA quantification method, and others.

Size of circulating DNA

A good portion of the cell-free DNA in plasma is resulting from apoptotic cells. As a result, a considerable percentage of this circulating nucleosomal DNA is known to be highly fragmented. The degree of fragmentation and the proportion of fragmented DNA relative to high molecular weight DNA however depends on several parameters like origin of the DNA (e.g. fetal, tumor, microbial DNA), health of the blood donor, blood sampling procedure, and handling of the sample.

The performance of many downstream applications consequently depends on the efficient isolation even of smallest DNA fragments (Chan *et al.* 2006, 2005, 2004, 2003; Deligezer *et al.* 2006; Giacona *et al.* 1998; Hanley *et al.* 2006; Hromadnikova *et al.* 2006; Jiang *et al.* 2006; Koide *et al.* 2005; Li *et al.* 2006, 2005, 2004; Wang *et al.* 2004).

According to this the NucleoSpin® Plasma XS purification system is designed for the efficient isolation of highly fragmented DNA in a range of 50 – 1000 bp. Within this range fragments are recovered with a similar high efficiency.

2.4 Handling of sample material

Several publications indicate strong influence of blood sampling, handling, storage and plasma preparation on DNA yield and DNA quality (Page et al. 2006; Sozzi et al. 2005; Chan et al. 2005; Lam et al. 2004; Jung et al. 2003). Therefore it is absolutely recommended to keep blood sampling procedure, handling, storage and plasma preparation method constant in order to achieve highest reproducibility.

Plasma can be isolated according to protocols described in literature (e.g. Chiu and Lo 2006; Birch *et al.* 2005) or according to the following recommendation:

Preparation of plasma from human EDTA blood

1 Centrifuge fresh blood sample for 10 min at 2,000 x g

2 Remove the plasma without disturbing sedimented cells

3 Freeze plasma at -20°C for storage upon DNA isolation

4 Thaw frozen plasma samples prior to DNA isolation and centrifuge for 3 min at ≥11,000 x g in order to remove residual cells, cell debris and particulate matter. Use the supernatant for DNA isolation.

2.5 Elution procedures

The recommended standard elution volume is 20 µl. A reduction of the elution volume to 5 – 15 µl will increase DNA concentration, the total DNA yield is decreased by this reduction however. An increase of the elution volume to 30 µl or more will only slightly increase total DNA yield but will reduce DNA concentration. Figure 1 (page 8) gives you a graphic description of the correlation between elution volume and DNA concentration and will thus help you to find the optimized elution volume for your individual application.

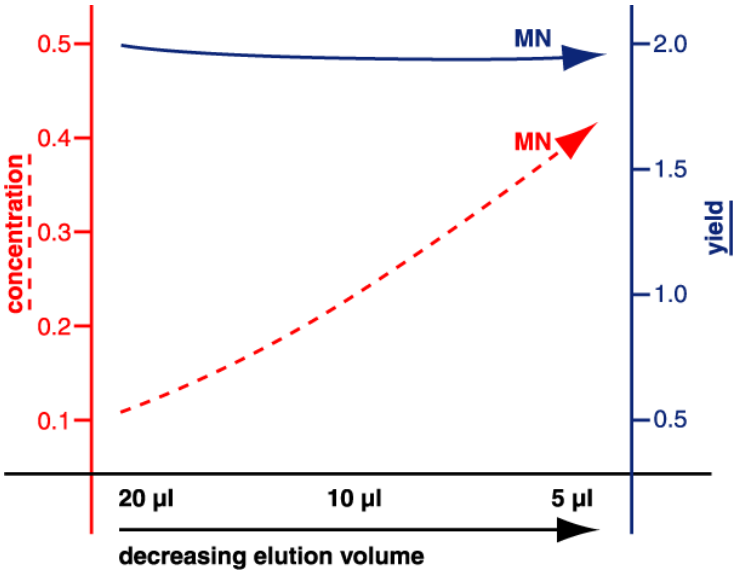


Fig. 1: Correlation between elution volume and DNA concentration (NucleoSpin Plasma® XS columns)

2.6 Removal of residual traces of ethanol for highest sensitivity

A reduction of the 20 µl standard elution volume will increase the concentration of residual ethanol in the eluate. For a 20 µl elution volumes a heat incubation of the elution fraction (incubate eluate with open lid for 8 min at 90°C) is recommended if the eluate comprises more than 20% of the final PCR volume in order to avoid an inhibition of sensitive downstream reactions. In this context, please mind the remarks below:

a) An Incubation of the elution fraction at higher temperatures will increase signal output in PCR. This is of importance especially if the template represents more than 20 % of the total PCR reaction volume (e.g. more than 4 µl eluate used as template in a PCR reaction with a total volume of 20 µl.

   The template may represent up to 40%* of the total PCR reaction volume, if the eluate is incubated at increased temperature as described

b) A volume of 20 µl used for elution will evaporate to 12 – 14 µl during a heat incubation for 8 min at 90°C. If a higher final volume is required, please increase the initial volume of elution buffer e.g. from 20 µl to 30 µl.

c) An incubation of the elution fraction for 8 min at 90°C will denature DNA. If non denatured DNA is required (like for downstream applications other than

* *The maximum percentage of template volume in a PCR reaction may vary depending on the robustness of the PCR system; 40% template volume were tested using LightCycler PCR (Roche) with the DyNAmo Capillary SYBR Green qPCR Kit (Finnzymes).*

PCR; e.g. ligation/cloning), we recommend an incubation for a longer time at a temperature below 80°C as most of the DNA has a melting point above 80°C. Suggestion: incubate for 17 min at 75°C.

d) The incubation of the eluate at higher temperatures may be adjusted according to Fig. 2. The incubation times and conditions shown will reduce an initial elution volume of 20 µl to about 12 – 14 µl and will effectively remove traces of ethanol as described above.

e) If the initial volume of elution buffer applied to the column is less than 20 µl, heat incubation times should be reduced in order to avoid complete dryness.

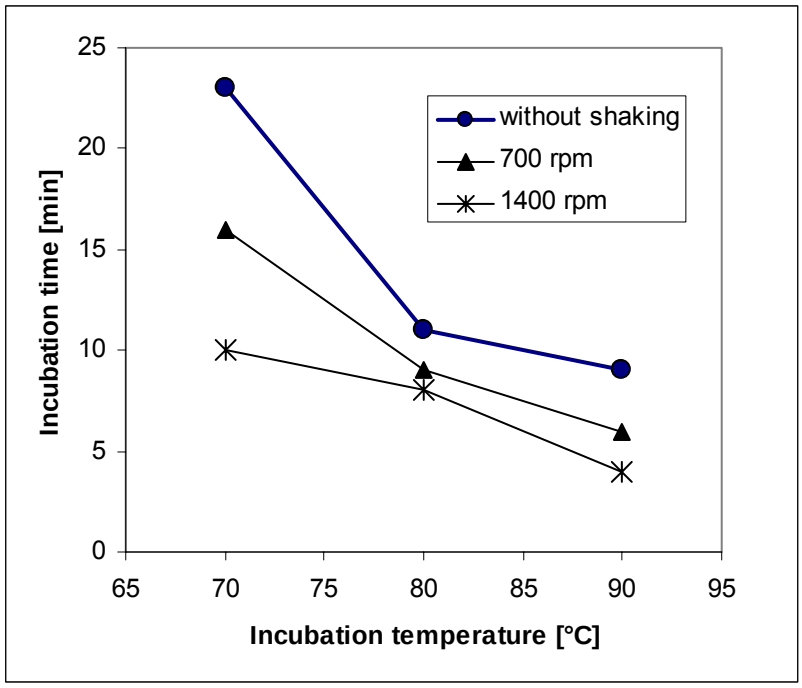


Fig. 2: Removal of residual ethanol from the elution fraction by heat treatment. In order to obtain highest PCR sensitivity, a heat incubation of the eluate is recommended. Heat incubation may be performed at temperatures of 70 – 90°C in a heat block with or without shaking. Effective conditions (temperature, time and shaking rate) for ethanol removal can be read from the diagram; an initial volume of 20 µl will evaporate to 12 - 14 µl during the described incubation.

2.7 Stability of isolated DNA

Due to the typically low DNA content in plasma and the resulting low total amount of isolated DNA, its fragmentation and the absence of DNase inhibitors (the elution buffer does NOT contain EDTA) the eluates should be placed on ice for short term and frozen at -20°C for long term storage.

3 Storage conditions and preparation of working solutions

Attention:

The binding buffer BB contains guanidine thiocyanate and ethanol! Wear gloves and goggles!

- All kit components can be stored at room temperature (20 - 25°C) and are stable up to one year.

Before starting any NucleoSpin® Plasma XS protocol prepare the following:

- Before first use of the kit, add Proteinase Buffer (PB) to dissolve lyophilized proteinase K as indicated (see bottle or table below). Proteinase K is stable at 4°C for up to 6 months. Storage at -20°C is recommended if the solution will not be used up during this period.

	NucleoSpin® Plasma XS		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740900.10**	**740900.50**	**740900.250**
Proteinase K (lyophilized)	6 mg	30 mg	2x 75 mg
	add 260 µl Proteinase Buffer	add 1.35 ml Proteinase Buffer	add to each vial 3.35 ml Proteinase Buffer

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® Plasma XS kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
BB	guanidine thiocyanate, + ethanol	Xn*	Flammable. Harmful by inhalation, in contact with skin and if swallowed.	R 10-20/21/22	S 7-13-16
WB	ethanol	F*	Highly flammable.	R 11	S 7-16
Proteinase K	Proteinase K, lyophilized	Xn Xi	Irritating to eyes, respiratory system and skin, may cause sensitization by inhalation	R 36/37/38-42	S 22-24-26-36/37

Risk Phrases

R 10	Flammable
R 11	Highly flammable
R 20/21/22	Harmful by inhalation, in contact with the skin and if swallowed
R 36/37/38	Irritating to eyes, respiratory system and skin
R 42	May cause sensitization by inhalation

Safety Phrases

S 7	Keep container tightly closed
S 13	Keep away from food, drink and animal feedstuffs
S 16	Keep away from sources of ignition – No Smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 26	Avoid contact with the eyes
S 36/37	Wear suitable protective clothing and gloves

* Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

5 Protocols

Equilibrate sample to room temperature (18°C – 25°C) and make sure that the sample is cleared from residual cells, cell debris,and particulate matter (e.g. by centrifugation of the plasma sample for 3 min at ≥11.000 x g).

For the High Sensitivity procedure: Set the thermal heating block to 75°C - 90°C for final ethanol removal (see section 2.6 for details).

5.1 High Sensitivity protocol for the isolation of DNA from plasma

The High Sensitivity procedure is recommended if highest DNA yield and concentration is required. The Rapid procedure (5.2) is recommended if shortest preparation time is required.

Optional: Spike addition

Pipet an appropriate DNA spike into the lid of a 1.5 ml reaction tube.

An appropriate DNA spike can be e.g. 20 µl of a solution containing one or several DNA fragments of 50 – 1000 bp with a concentration of 1 ng/µl per fragment for subsequent analysis of DNA recovery (e.g. via Agilent´s Bioanalyzer 2100).

1 Prepare sample

Add **240 µl plasma** to a microcentrifuge tube (not provided).

240 µl plasma

Less than 240 µl may be used. Adopt the binding buffer volume accordingly (see below).

1a *Optional: Proteinase K treatment*

Add **20 µl Proteinase K** to the plasma sample, mix, and incubate at 37°C for 10 min.

Optional:

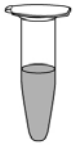
+ 20 µl Proteinase K

Depending on the plasma sample and the PCR conditions, the proteinase treatment of the plasma sample provokes a increase of the PCR signal of 0.5 - 1.5 cycles, i.e. the cycle threshold (Ct-value) / crossing point (Cp-value) is reached 0.5 - 1.5 cycles earlier. The proteinase treatment may however alter the ratio of high to low molecular weight DNA.

2 Adjust DNA binding conditions

Add **360 µl binding buffer BB.**

If less than 240 µl plasma is used, adjust the binding buffer volume accordingly. A ratio of 1:1.5 (v/v) for plasma and binding buffer has to be ensured.



+ 360 µl BB

3 Mix sample

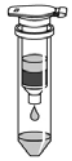
Invert the tube **3 x** and vortex for **3 sec**. Centrifuge the tube briefly to clean the lid.

Make sure to invert the tube especially if working with a spike in the tube lid: vortexing alone does not securely flush liquid from the lid into the tube.

mix sample

4 Bind DNA

For each sample, **load** the mixture (**600 µl**) to a NucleoSpin® Plasma XS column placed in a 2 ml collecting tube.



load lysate

Centrifuge at **2,000 x g** for **30 sec,** increase centrifuge force to **11,000 x g** for further **5 sec**. Discard collecting tube with flow-through and place column into new collecting tube (provided).

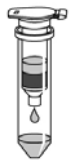
30 sec
2,000 × g

The maximal column volume is approx. 600 µl. Do not apply a higher volume in order to avoid spillage. If larger plasma sample volumes have to be processed, the loading step may be repeated. Be aware of an increased risk of membrane clogging in case of multiple column loading steps. If the solution has not completely passed the column, centrifuge for an additional 60 sec at 11,000 x g.

5 sec
11,000 × g

5 Wash silica membrane (1st wash)

Add **500 µl wash buffer WB** to the column. Centrifuge at **11,000 x g** for **30 sec.** Discard collecting tube with flow-through and place column into new collecting tube (provided).

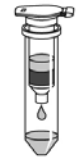


+ 500 µl WB

30 sec
11,000 x g

6 **Wash** (2nd wash) **and dry silica membrane**

Add **250 µl wash buffer WB** to the column. Centrifuge at **11,000 x g** for **3 min**. Discard collecting tube with flow-through and place column into a 1.5 ml microcentrifuge tube for elution (not provided).



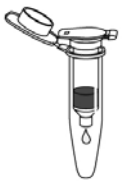
+ 250 µl WB

3 min
11,000 x g

7 **Elute DNA**

Add **20 µl Elution Buffer** to the column. Centrifuge at **11,000 x g** for **30 sec**.

Elution volume may be varied from approximately 5 – 30 µl. For a correlation of elution volume, DNA concentration and DNA amount eluted from the column see section 2.5.



+ 20 µl
Elution Buffer

30 sec
11,000 x g

8 **Removal of residual ethanol**

Incubate elution fraction with open lid for **8 min** at **90°C**.

See section 2.6 for further comments and alternative incubation times and temperatures for a removal of residual ethanol.

8 min
90°C

5.2 Rapid protocol for the isolation of DNA from plasma

The rapid procedure represents a good compromise between DNA yield and concentration as well as ease and speed of nucleic acid extraction.

Optional: Spike addition

Pipet an appropriate DNA spike into the lid of a 1.5 ml reaction tube.

An appropriate DNA spike can be e.g. 20 µl of a solution containing one or several DNA fragments of 50 – 1000 bp with a concentration of 1 ng/µl per fragment for subsequent analysis of DNA recovery (e.g. via Agilent´s Bioanalyzer 2100).

1 Prepare sample

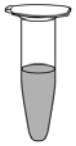
Add **200 µl plasma** to a microcentrifuge tube (not provided).

200 µl plasma

Less than 200 µl may be used. Adopt the binding buffer volume accordingly (see below).

2 Adjust DNA binding conditions

Add **300 µl binding buffer BB.**



+ 300 µl BB

If less than 200 µl plasma is used, adjust the binding buffer volume accordingly. A ratio of 1:1.5 (v/v) for plasma and binding buffer has to be ensured.

3 Mix sample

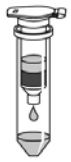
Invert the tube **3x** and vortex for **3 sec**. Centrifuge the tube briefly to clean the lid.

mix sample

Make sure to invert the tube especially if working with a spike in the tube lid: vortexing alone does not securely flush liquid from the lid into the tube.

4 Bind DNA

For each sample, **load** the mixture (**500 µl**) to a NucleoSpin® Plasma XS column placed in a 2 ml collecting tube.



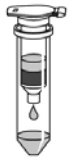
load lysate

Centrifuge at **11,000 x g** for **30 sec**. Discard collecting tube with flow-through and place column into new collecting tube (provided).

30 sec
11,000 × g

The maximal column volume is approx. 600 µl. Do not apply a higher volume in order to avoid spillage. If larger plasma sample volumes have to be processed, the loading step may be repeated. Be aware of an increased risk of membrane clogging in case of multiple column loading steps. If the solution has not completely passed the column, centrifuge for an additional 60 sec at 11,000 x g.

5 Wash silica membrane (1st wash)

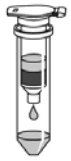


+ 500 µl WB

Add **500 µl wash buffer WB** to the column. Centrifuge at **11,000 x g** for **30 sec.** Discard collecting tube with flow-through and place column into new collecting tube (provided).

30 sec
11,000 x g

6 Wash (2nd wash) **and dry silica membrane**

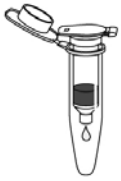


+ 250 µl WB

Add **250 µl wash buffer WB** to the column. Centrifuge at **11,000 x g** for **3 min**. Discard collecting tube with flow-through and place column into a 1.5 ml microcentrifuge tube for elution (not provided).

3 min
11,000 x g

7 Elute DNA



+ 20 µl
Elution Buffer

Add **20 µl Elution Buffer** to the column. Centrifuge at **11,000 x g** for **30 sec**.

30 sec
11,000 x g

Elution volume may be varied from approximately 5 – 30 µl. For a correlation of elution volume, DNA concentration and DNA amount eluted from the column see section 2.5.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Low DNA yield	*Low DNA content of the sample* • The content of cell-free DNA in human plasma may vary over several orders of magnitude. DNA contents from approximately 0.1 – 1000 ng DNA per ml of plasma have been reported (see remarks in section 2.3).
Column clogging	*Sample contains residual cell debris or cells* • The plasma sample may have contained residual cells or cell debris. Make sure to use only clear plasma samples (see remarks in section 2.4).
No increase of PCR signal despite of an increased volume of eluate used as template in PCR	*Residual ethanol in eluate* • Please see the detailed description of removal of residual traces of ethanol in section 2.6.
Discrepancy between A_{260} quantification values and PCR quantification values	*Silica abrasion from the membrane* • Due to the typically low DNA content in plasma and the resulting low total amount of isolated DNA, a DNA quantification via A_{260} absorption measuerment is often hampered due to the low sensitivity of the absorption measuement. When performing absorption measurements close to the detection limit of the photometer, the measurement may be influenced by minor amounts of silica abrasion. In order to prevent incorrect A_{260}-quantification of small DNA amounts centrifuge the eluate for 30 sec. at >11.000 x g and take an aliquot for measuement without disturbing any sediment. Alternatively, use a silica abrasion insensitive DNA quantification method (e.g. PicoGreen fluorecent dye).

	Measurement not in the range of photometer detection limit
Unexpected $A_{260/280}$ ratio	• In order to obtain a significant $A_{260/280}$ ratio it is necessary that the initially measured A_{260} and A_{280} values are significantly above the detection limit of the photometer used. An A_{280} value close to the background noise of the photometer will cause unexpeced $A_{260/280}$ ratios.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® Plasma XS	740900.10	10 preps
NucleoSpin® Plasma XS	740900.50	50 preps
NucleoSpin® Plasma XS	740900.250	250 preps
NucleoSpin® collecting tubes (2 ml)	740600	1000

6.3 Literature

Birch L, English CA, O'Donoghue K, Barigye O, Fisk NM, Keer JT.: Accurate and robust quantification of circulating fetal and total DNA in maternal plasma from 5 to 41 weeks of gestation. Clin Chem. 2005 Feb;51(2):312-20. Epub 2004 Dec 17.

Chan KC, Lo YM.: Clinical applications of plasma Epstein-Barr virus DNA analysis and protocols for the quantitative analysis of the size of circulating Epstein-Barr virus DNA. Methods Mol Biol. 2006;336:111-21.

Chan KC, Yeung SW, Lui WB, Rainer TH, Lo YM.: Effects of preanalytical factors on the molecular size of cell-free DNA in blood. Clin Chem. 2005 Apr;51(4):781-4. Epub 2005 Feb 11.

Chan KC, Zhang J, Chan AT, Lei KI, Leung SF, Chan LY, Chow KC, Lo YM. Molecular characterization of circulating EBV DNA in the plasma of nasopharyngeal carcinoma and lymphoma patients. Cancer Res. 2003 May 1;63(9):2028-32.

Chan KC, Zhang J, Hui AB, Wong N, Lau TK, Leung TN, Lo KW, Huang DW, Lo YM. Size distributions of maternal and fetal DNA in maternal plasma. Clin Chem. 2004 Jan;50(1):88-92.

Chiu RW, Lo YM.: Noninvasive prenatal diagnosis by analysis of fetal DNA in maternal plasma. Methods Mol Biol. 2006;336:101-9.

Chiu TW, Young R, Chan LY, Burd A, Lo DY. Plasma cell-free DNA as an indicator of severity of injury in burn patients. Clin Chem Lab Med. 2006;44(1):13-7.

Chun FK, Muller I, Lange I, Friedrich MG, Erbersdobler A, Karakiewicz PI, Graefen M, Pantel K, Huland H, Schwarzenbach H. Circulating tumour-associated plasma DNA represents an independent and informative predictor of prostate cancer. BJU Int. 2006 Sep;98(3):544-8.

Deligezer U, Erten N, Akisik EE, Dalay N. Circulating fragmented nucleosomal DNA and caspase-3 mRNA in patients with lymphoma and myeloma. Exp Mol Pathol. 2006 Feb;80(1):72-6. Epub 2005 Jun 15.

Fatouros IG, Destouni A, Margonis K, Jamurtas AZ, Vrettou C, Kouretas D, Mastorakos G, Mitrakou A, Taxildaris K, Kanavakis E, Papassotiriou I. Cell-free plasma DNA as a novel marker of aseptic inflammation severity related to exercise overtraining. Clin Chem. 2006 Sep;52(9):1820-4. Epub 2006 Jul 13.

Giacona MB, Ruben GC, Iczkowski KA, Roos TB, Porter DM, Sorenson GD. Cell-free DNA in human blood plasma: length measurements in patients with pancreatic cancer and healthy controls. Pancreas. 1998 Jul;17(1):89-97.

Hanley R, Rieger-Christ KM, Canes D, Emara NR, Shuber AP, Boynton KA, Libertino JA, Summerhayes IC. DNA integrity assay: a plasma-based screening tool for the detection of prostate cancer. Clin Cancer Res. 2006 Aug 1;12(15):4569-74.

Hromadnikova I, Zejskova L, Doucha J, Codl D. Quantification of fetal and total circulatory DNA in maternal plasma samples before and after size fractionation by agarose gel electrophoresis. DNA Cell Biol. 2006 Nov;25(11):635-40.

Jiang WW, Zahurak M, Goldenberg D, Milman Y, Park HL, Westra WH, Koch W, Sidransky D, Califano J. Increased plasma DNA integrity index in head and neck cancer patients. Int J Cancer. 2006 Dec 1;119(11):2673-6.

Jung M, Klotzek S, Lewandowski M, Fleischhacker M, Jung K.: Changes in concentration of DNA in serum and plasma during storage of blood samples. Clin Chem. 2003 Jun;49(6 Pt 1):1028-9.

Koide K, Sekizawa A, Iwasaki M, Matsuoka R, Honma S, Farina A, Saito H, Okai T. Fragmentation of cell-free fetal DNA in plasma and urine of pregnant women. Prenat Diagn. 2005 Jul;25(7):604-7.

Lam NY, Rainer TH, Chiu RW, Lo YM.: EDTA is a better anticoagulant than heparin or citrate for delayed blood processing for plasma DNA analysis. Clin Chem. 2004 Jan;50(1):256-7.

Lazar L, Nagy B, Ban Z, Nagy GR, Papp Z. Presence of cell-free fetal DNA in plasma of women with ectopic pregnancies. Clin Chem. 2006 Aug;52(8):1599-601. Epub 2006 Jun 1.

Li Y, Di Naro E, Vitucci A, Zimmermann B, Holzgreve W, Hahn S. Detection of paternally inherited fetal point mutations for beta-thalassemia using size-fractionated cell-free DNA in maternal plasma. JAMA. 2005 Feb 16;293(7):843-9. Erratum in: JAMA. 2005 Apr 13;293(14):1728.

Li Y, Holzgreve W, DI Naro E, Vitucci A, Hahn S. Cell-free DNA in maternal plasma: is it all a question of size? Ann N Y Acad Sci. 2006 Sep;1075:81-7.

Li Y, Holzgreve W, Page-Christiaens GC, Gille JJ, Hahn S. Improved prenatal detection of a fetal point mutation for achondroplasia by the use of size-fractionated circulatory DNA in maternal plasma--case report. Prenat Diagn. 2004 Nov;24(11):896-8.

Li Y, Wenzel F, Holzgreve W, Hahn S. Genotyping fetal paternally inherited SNPs by MALDI-TOF MS using cell-free fetal DNA in maternal plasma: influence of size fractionation. Electrophoresis. 2006 Oct;27(19):3889-96.

Li Y, Zimmermann B, Rusterholz C, Kang A, Holzgreve W, Hahn S. Size separation of circulatory DNA in maternal plasma permits ready detection of fetal DNA polymorphisms. Clin Chem. 2004 Jun;50(6):1002-11. Epub 2004 Apr 8.

Page K, Powles T, Slade MJ, DE Bella MT, Walker RA, Coombes RC, Shaw JA. The Importance of Careful Blood Processing in Isolation of Cell-Free DNA. Ann N Y Acad Sci. 2006 Sep; 1075:313-317.

Rainer TH, Lam NY, Man CY, Chiu RW, Woo KS, Lo YM. Plasma beta-globin DNA as a prognostic marker in chest pain patients. Clin Chim Acta. 2006 Jun;368(1-2):110-3. Epub 2006 Feb 14.

Rhodes A, Wort SJ, Thomas H, Collinson P, Bennett ED. Plasma DNA concentration as a predictor of mortality and sepsis in critically ill patients. Crit Care. 2006;10(2):R60.

Schmidt B, Weickmann S, Witt C, Fleischhacker M. Improved method for isolating cell-free DNA. Clin Chem. 2005 Aug;51(8):1561-3.

Sozzi G, Roz L, Conte D, Mariani L, Andriani F, Verderio P, Pastorino U.: Effects of prolonged storage of whole plasma or isolated plasma DNA on the results of circulating DNA quantification assays. J Natl Cancer Inst. 2005 Dec 21;97(24):1848-50.

Wang M, Block TM, Steel L, Brenner DE, Su YH. Preferential isolation of fragmented DNA enhances the detection of circulating mutated k-ras DNA. Clin Chem. 2004 Jan;50(1):211-3.

6.4 Product use restriction / warranty

NucleoSpin® Plasma XS kit components were developed, designed, distributed and sold **for RESEARCH PURPOSES ONLY**. They are suitable ***for IN – VITRO USES only.*** No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoSpin® Plasma XS** kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

MACHEREY-NAGEL does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; against defects in products or components not manufactured by MACHEREY-NAGEL, or against damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Please contact:

MACHEREY-NAGEL Germany
Tel.: +49 (0) 2421 969 270
e-mail: TECH-BIO@mn-net.com

Last updated 12/2006, Rev 02